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ORIGINAL PAPER

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Formation of 3-chloropropane-1,2-diol in systems simulating processed foods

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Abstract Formation of 3-chloropropane-1,2-diol (3-MCPD) from the precursors glycerol, triolein and soy lecithin in the presence of sodium chloride was studied. The precursors were reacted with sodium chloride in an emulsion stabilised with an emulsifier under conditions which modelled the thermal treatment of foods during processing. Three sets of experiments were carried out aimed to monitor the influence of various factors (NaCl, water content and temperature) on the yield of 3-MCPD. The formation of 3-MCPD strongly depended on the concentration of NaCl and reached a maximum level at about 4–7% NaCl. The highest amount of 3-MCPD was formed in media containing approximately 13–17% water. The amount of 3-MCPD increased with increasing temperature over the range 100–230 °C and reached its highest value at 230 °C. The production of 3-MCPD was also followed in models very closely related to selected foods which had been shown to have a high potential to yield 3-MCPD during processing (salami, beefburgers, processed cheese, biscuits, crackers, doughnuts). The highest levels of 3-MCPD were formed in models simulating salami as they had the highest content of both fat and salt of all the samples. The lowest amount of 3-MCPD was formed in the models simulating biscuits and crackers as they had a low salt content and, at the same time, their water content was below the optimum level.

Keywords 3-Chloropropane-1,2-diol · 3-Monochloropropane-1,2-diol · 3-MCPD · Processed foods

Introduction

Acid-hydrolysed vegetable protein (acid-HVP) commonly manufactured by hydrolysis of proteinaceous vegetable materials using hydrochloric acid at elevated temperatures and at appropriate pressure was introduced in 1886 and has been used regularly since that time as such and as a seasoning ingredient in a variety of processed and pre-prepared foods such as soups, sauces, bouillon cubes and soy sauce. It was recognised in 1978 that the procedure used to manufacture the acid-HVP resulted in the formation of a new class of food contaminants known as chloropropanols [1]. The most common chloropropanol 3-MCPD (3-chloropropane-1,2-diol) was identified in model experiments with lipids hydrolysed by hydrochloric acid in 1979 [2, 3] and found in acid-HVP in 1981 [4]. Its isomer 2-MCPD (2-chloropropane-1,3-diol) was identified in HVP in 1987 [5]. Recently, (*R*)-3-MCPD and (*S*)-3-MCPD were found in acid-HVP as a racemic mixture [6].

Toxicological studies on 3-MCPD prompted the European Commission to set a maximum level of 0.02 mg/kg for 3-MCPD in soy sauce and HVP sold within the European Community with effect from 5th April 2002 [7].

It has been established that hydrochloric acid and lipids occurring in the raw materials used for acid-HVP production are the precursors to the chloropropanols [1]. Model experiments with glycerol [2, 5], triacylglycerols [2, 3, 5], phospholipids [5, 8] and lipids isolated from the raw materials [8] have clearly shown that 3-MCPD is predominantly formed in acid-HVP by reaction of hydrochloric acid with residual lipids associated with the proteinaceous materials used in their production. It has been shown that the most important precursors of chloropropanols are triacylglycerols and, to a smaller extent, phospholipids and glycerol in decreasing order. Corresponding reaction mechanisms have been presented [5].

Recent studies [9, 10] have demonstrated the presence of 3-MCPD and 2-MCPD at levels of 0.01–0.03 mg/kg in

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a range of foods and food ingredients, where they evidently arise from lipids and naturally present or intentionally added sodium chloride as a result of processing. These studies have been mainly focused on meat (salami, beefburgers), and cereal products (malt, malt extract). Domestic cooking of foods has also been shown to result in elevated levels of 3-MCPD in, e.g. toasted biscuits and breads, doughnuts, grilled cheeses and fried batters [11].

Thermal processing is a common method of food preparation that imparts desired sensory characteristics of food (flavour, colour and texture). During frying (as well as during some other thermal processes) at approximately 190 °C, fats thermally and oxidatively decompose to give a substantial number of various products. Water and steam hydrolyse triacylglycerols and phospholipids, producing diacylglycerols, monoacylglycerols, free fatty acids, glycerol and other products. Other non-volatile polar products include partial acylglycerols containing chain scission products, triacylglycerol oligomers and oxidized triacylglycerols. Water partly evaporates, especially in the surface layer of food. Glycerol partially decomposes and partially evaporates, since it volatilises above 150 °C, and the reaction equilibrium is thus shifted in favour of the hydrolysis products. The extent of hydrolysis is a function of various factors, such as oil temperature, interface area between oil and the aqueous phase, amount of water and steam (since water hydrolyses lipids more quickly than steam) [12].

Kinetic data on reactions of chlorides with glycerol and lipids in systems of low water activity and at high temperatures (such as in the surface layers of thermally treated foods) are not available in the literature but analogous reactions may proceed in aprotic solvents and in the gas phase [13]. It is known that solvents can strongly affect the properties of molecules in solutions by hydration (generally solvation) effects and in non-polar media (e.g. fats and oils) or media of low water activity (e.g. emulsions) as well as in the gas phase the solvation effects are totally eliminated or substantially reduced. In solutions, small negatively charged nucleophiles (e.g. OH^- and Cl^-) are solvated by polar protic solvents (for example hydrogen-bonded to water), the shell of solvent molecules constitutes a barrier between it and the substrate (i.e. lipid molecule) and decreases the rate of nucleophilic substitutions. Evidence for this is that many nucleophilic substitutions with small negatively charged nucleophiles are much more rapid in aprotic polar solvents (where the ions are completely free, unsolvated and unassociated) than in protic ones. This enhanced reactivity of chlorides due to polarity of the medium is further enhanced by temperature that often exceeds 200 °C.

It is evident that further studies are needed to investigate the formation of 3-MCPD from lipids and sodium chloride under conditions more typical of baking, roasting and toasting processes. Therefore, the aim of this research was to study the formation of 3-MCPD in models using systems with low water activity simulating surface layers of foods, systems with various classes of lipids and sodium chloride as the precursors of 3-MCPD and heated to high temperatures corresponding to those encountered during the thermal processing of foods.

To simulate the formation of 3-MCPD in the surface layers of thermally processed foods containing naturally present or intentionally added salt, experiments were carried out using mixtures of the major recognised 3-MCPD precursors (glycerol/triolein/lecithin) with NaCl and water. An inert emulsifier was added to make a dispersed system of these immiscible substances. Three sets of experiments were carried out with the aim to monitor the influence of various factors (NaCl content, water content, temperature) on the yield of 3-MCPD.

Materials and methods

Chemicals

3-Chloropropane-1,2-diol (98%) was obtained from Merck (Germany), phenylboronic acid was from Fluka Chemie (Switzerland). Glycerol, Tween 80, sodium chloride and paraffin were from Lachema (Czech Republic). Triolein (61%) was produced by Downs Development Chemicals (UK). Its composition determined by HPLC [14] was: 95.1% triacylglycerols, 3.6% diacylglycerols, 0.3% monoacylglycerols, 0.5% polymers, 0.5% free fatty acids. Crude soy lecithin was supplied by Setuza a.s. (Czech Republic) and purified by extraction with acetone. The phosphorus content of the purified sample was determined by atomic absorption spectroscopy (Central Laboratories of ICT Prague) and found to be 29.2 g/kg. This value was re-calculated according to Marmer [15] as lecithin (i.e. *sn*-1,2-dioleoyl-3-phosphocholine) of 93.6% purity.

Model systems

Reaction of 3-MCPD precursors with sodium chloride. The respective 3-MCPD precursor, i.e. glycerol, triolein or lecithin (200 mg each), NaCl (10 mg, i.e. 3.47 wt% or 5 wt% with respect to the 3-MCPD precursor) and an inert emulsifier (Tween 80, 30 mg) were placed in a 5-ml glass tube, and 48 μl water was added. The tube was sealed and heated in an oven at 200 °C for 30 min and then cooled to room temperature. The cold tube was opened and 0.2 ml of the internal standard propane-1,2-diol (200 μg) in water were added. The tube contents were transferred to a separating funnel and the tube was rinsed with three portions of hexane (one 4-ml portion and two 3-ml portions) and the washings transferred to the separating funnel. The tube was then rinsed with 2 ml of water and these washings were also added to the separating funnel and its contents shaken. The aqueous phase was collected in a 25-ml distillation flask. The washing and extraction steps were repeated with three × 2-ml portions of water. The final aqueous extract was evaporated to dryness. The residue was dissolved in 2 ml of 20% sodium chloride solution and used for determination of 3-MCPD by gas-liquid chromatography.

Effect of sodium chloride. Experiments with mixtures consisting of 3-MCPD precursor, i.e. glycerol, triolein or lecithin (200 mg), Tween 80 (30 mg), water (16.67 wt%) and NaCl (0%, 0.72%, 1.77%, 3.47%, 5.10% and 6.67 wt%, i.e. 0 wt%, 1 wt%, 2.5 wt%, 5 wt%, 7.5 wt% and 10 wt% when expressed to total solids) were carried out at 200 °C for 30 min.

Effect of water. Mixtures consisting of 3-MCPD precursors (glycerol, triolein or lecithin, 200 mg), emulsifier Tween 80 (30 mg), NaCl (3.47 wt%) and water (0.00 wt%, 4.76 wt%, 9.09 wt%, 13.04 wt%, 16.67 wt%, 20.00 wt%, i.e. 0%, 5%, 10%, 15%, 20%, 25% if calculated to total solids) were carried out at 200 °C for 30 min.

Effect of temperature. Systems consisted of 3-MCPD precursor, i.e. glycerol, triolein or lecithin (200 mg), Tween 80 (30 mg), 3.47% NaCl and 16.67% water, heated at temperatures of 100 °C, 140 °C, 170 °C, 200 °C and 230 °C for 30 min. The samples were extracted as described above.

Methods

Determination of 3-chloropropane-1,2-diol. The method of Plantinga et al. [16] was used with slight modifications. Some residue (2 ml) dissolved in 2 ml of 20% NaCl solution (see above) was heated for 20 min at 90 °C with 0.2 ml phenylboronic acid in acetone (250 mg/ml) in a 25-ml flask. The flask was cooled and the 3-MCPD extracted into 2 ml hexane by shaking. Some (1 μl) of the sample (hexane layer) was analysed by GLC at a split rate of 2:1 using an SPB-I fused silica capillary column (30 m × 0.20 mm i.d., 0.80 μm film thickness, Supelco, USA) fitted to a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionisation detector. The oven temperature was initially set to 80 °C, raised to 300 °C at a rate of 5 °C/min and kept at 300 °C for 16 min. The injector and detector port temperatures were set to 250 °C and 300 °C, respectively. The helium carrier gas flow rate was 0.5 ml/min. Two parallel determination of each sample were made. The results were expressed in mg 3-MCPD per kg of precursor and in μmole of 3-MCPD per mole of precursor.

Results and discussion

Effect of sodium chloride

The results of experiments aimed to study the formation of 3-MCPD from glycerol, triolein and lecithin influenced by the amount of NaCl are presented in Fig. 1 (in mg/kg precursor). As can be seen, the quantity of 3-MCPD formed depended strongly on the concentration of NaCl and reached a maximum value at about 3–7% NaCl. It is probable that in the presence of higher amounts of NaCl, even higher amounts of 3-MCPD would form. One kg of glycerol gave rise to about 6 mg 3-MCPD (in models with 3.5–6.7% NaCl), while about 2 mg 3-MCPD were formed per kg of lecithin and 0.5 mg 3-MCPD per kg of triolein. A different picture was obtained by plotting the corresponding molar concentration of 3-MCPD and its precursors against the amount of NaCl added. In this case, the most potent precursor of 3-MCPD was lecithin followed by glycerol and triolein.

Analogous results were obtained for the formation of 2-MCPD. The amount of 2-MCPD produced from glycerol was about 1 mg/kg, i.e. approximately six times lower than for 3-MCPD under the same conditions. Correspondingly lesser amounts of 2-MCPD were formed from lecithin and triolein.

Effect of water

The amount of water present in the reaction mixture also affected the formation of 3-MCPD from its precursors. To study this, model systems with glycerol/triolein/lecithin containing different amounts of water were evaluated. The greatest amount of 3-MCPD formed from glycerol and lecithin in media containing approximately 13–17% water (Fig. 2).

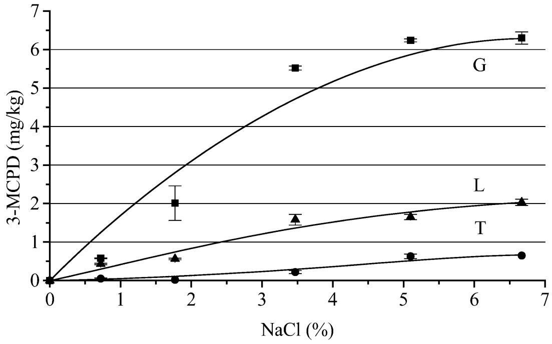

Fig. 1 Formation of 3-MCPD (mg/kg) influenced by NaCl amount (16.67% water, 200 °C). G=glycerol, T=triolein, L=lecithin

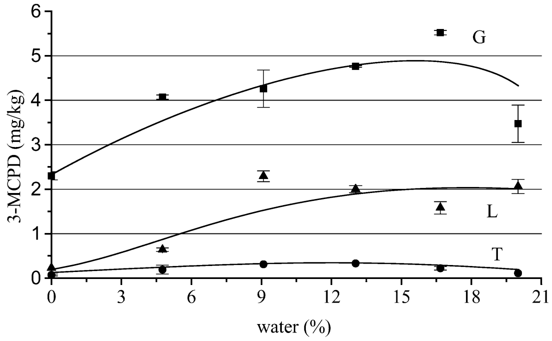

Fig. 2 Effect of water on 3-MCPD content (3.47% NaCl, 200 °C). G=glycerol, T=triolein, L=lecithin

It can be seen that in the systems with no water added (where the hydrolysis of the acyl group cannot proceed) the direct nucleophilic substitution of the hydroxyl group in glycerol by chloride ion to form 3-MCPD seemed to be slightly more facile than the direct nucleophilic substitution of the acyl groups in, e.g. triacylglycerols or phospholipids.

In systems containing water, acylglycerols and phospholipids can also be first hydrolysed to partial acylglycerols and further to glycerol that then reacts with chloride ions leading to the formation of 3-MCPD.

Effect of temperature

The influence of temperature on the formation of 3-MCPD was studied in the range 100–230 °C. The results illustrating the formation of 3-MCPD are given in Fig. 3. It can be seen that the amount of 3-MCPD increased with increasing temperature and reached the highest value at 230 °C. The greatest amount of 3-MCPD was again formed from glycerol followed by lecithin and triolein in that order.

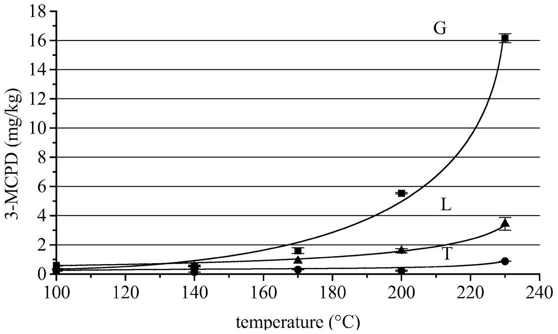

Fig. 3 Influence of temperature on 3-MCPD formation (16.67% water, 3.47% NaCl). G=glycerol, T=triolein, L=lecithin

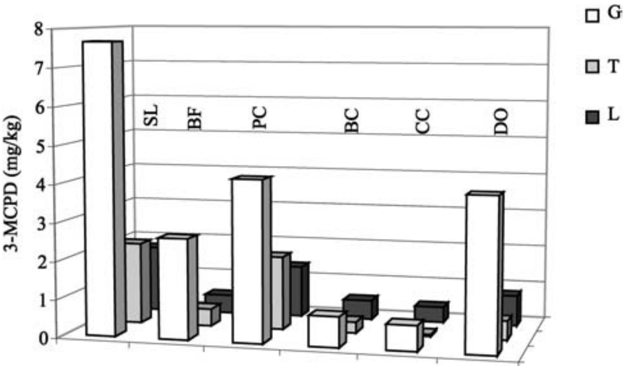

Fig. 4 Formation of 3-MCPD (mg/kg) in models simulating selected foods. SL=salami, BF=beefburgers raw, PC=processed cheese, BC=biscuits, CC=cream crackers, DO=doughnuts, G=glycerol, T=triolein, L=lecithin

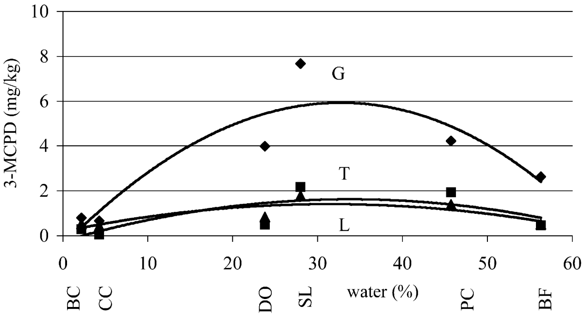

Fig. 5 Influence of water on 3-MCPD formation in models simulating selected foods. SL=salami, BF=beefburgers raw, PC=processed cheese, BC=biscuits, CC=cream crackers, DO=doughnuts, G=glycerol, T=triolein, L=lecithin

Models closely related to foods

The aim of this part of research was to study the formation of 3-MCPD in models very closely related to foods selected as those having a high potential to produce 3-MCPD during processing. The foods have been chosen to cover wide ranges of water (2–56%), fat (16–45%) and salt (0.6–4%) levels.

The models were again mixtures of water, fat and salt, with an inert material (paraffin) added to bring the total weight of each mixture to 280 mg. Details of the composition of the individual models are given in Table 1. Each of the mixtures was heated at 200 °C for 30 min.

It is evident (Fig. 4) that the major precursor of 3-MCPD was glycerol when expressed in units of weight. On the other hand, the major precursors of 3-MCPD were the lipids (triolein and lecithin) when the amount of these precursors was expressed in molar units.

The highest content of 3-MCPD (mg per kg of precursor) was found in models simulating salami. These had the highest content of both fat and salt of all the studied commodities. The plot of 3-MCPD concentration against the amount of water present in the models showed that the maximum amount of 3-MCPD was formed in models having a water content of about 30% (Fig. 5). In salami, of all the precursors, glycerol produced the greatest amount of 3-MCPD (7.7 mg/kg), more than three times that formed from triolein (2.2 mg/kg) and lecithin (1.8 mg/kg).

The smallest amount of 3-MCPD (mg per kg of precursor) was formed in the models simulating biscuits and cream crackers. These models had a low salt content and, at the same time, a water content below the optimum level of about 12%. Again, glycerol was the major producer of 3-MCPD (0.7 mg/kg), while triolein produced considerably less 3-MCPD (0.1 mg/kg). Lecithin produced greater amounts of 3-MCPD than triolein (0.4 mg/kg) but less than glycerol.

The influence of water on the formation of 3-MCPD can be further demonstrated in doughnuts and biscuits. Both of these products had almost the same fat and salt content but they differed in their water content. Under the experimental conditions, addition of glycerol produced approximately five times more 3-MCPD in systems modelling doughnuts (containing 23.8% water) in comparison with systems modelling biscuits (having only 2.2% water, which is far from the optimum).

Table 1 Chemical composition of models simulating real foods

Food product	Water	Glycerol/triolein/lecithin	Salt	Paraffin	Total solids
	(μl)	(mg)	(mg)	(mg)	(mg)
Salami	78	127	11	64	280
Beefburgers raw	158	57	4	61	280
Processed cheese	128	76	5	71	280
Biscuits	6	59	3	212	280
Cream crackers	12	46	4	218	280
Doughnuts	67	61	2	150	280

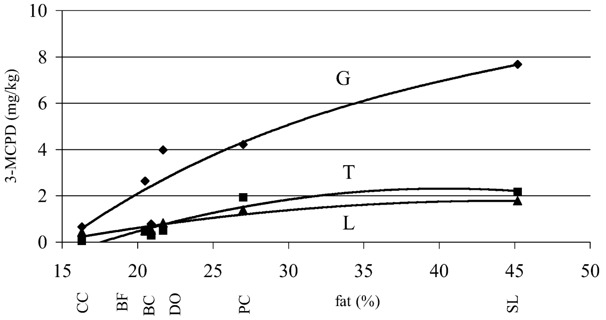

Fig. 6 Influence of fat on 3-MCPD formation in models simulating selected foods. SL=salami, BF=beefburgers raw, PC=processed cheese, BC=biscuits, CC=cream crackers, DO=doughnuts, G=glycerol, T=triolein, L=lecithin

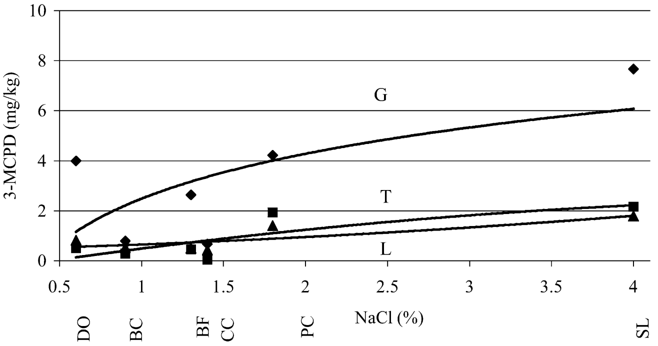

Fig. 7 Influence of salt on 3-MCPD formation in models simulating selected foods. SL=salami, BF=beefburgers raw, PC=processed cheese, BC=biscuits, CC=cream crackers, DO=doughnuts, G=glycerol, T=triolein, L=lecithin

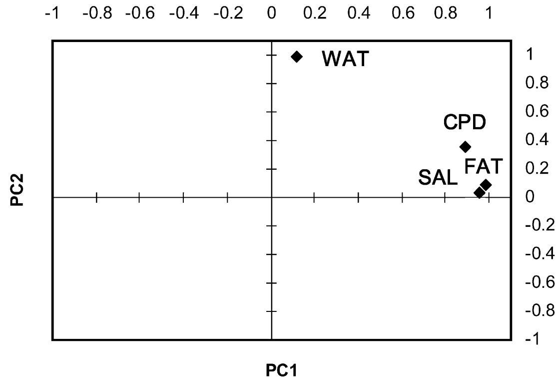

Fig. 8 Saturations of variables plotted in PC1 and PC2. WAT=water, FAT=fat, SAL=salt, CPD=3-MCPD

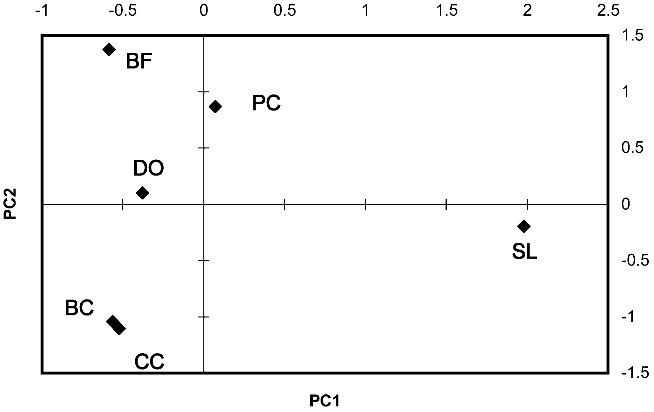

Fig. 9 Sample scores plotted in PC1 and PC2. SL=salami, BF=beefburgers raw, PC=processed cheese, BC=biscuits, CC=cream crackers, DO=doughnuts

A comparison of the 3-MCPD amounts formed, e.g. from glycerol in doughnut models (4.0 mg/kg) and in beefburger models (2.6 mg/kg) again showed that the water content (23.8% in doughnuts and 56.3% in beefburgers) when greater than the optimum, reduced the amount of 3-MCPD formed even if the salt content was greater (0.6% in doughnuts, 4.0% in beefburgers). The content of water present in the models played a more prominent role when glycerol was used and a less pronounced role in the case where lipids were employed as the precursors of 3-MCPD. Glycerol gave rise to 3-MCPD even in systems without water (Fig. 2).

Figure 6 shows that the amount of 3-MCPD formed with increasing fat content in the models. Similar results were obtained for plotting the 3-MCPD content against the amount of salt in the models (Fig. 7). The 3-MCPD levels found in real foods processed under the same temperatures must, of course, be lower in comparison with those in the models described. In real foods, only the surface layers reach the optimum (sufficiently low) water content and sufficiently high temperature. In models, the water content and temperature were the same throughout the whole sample.

The formation of 3-MCPD is a multivariate problem as it depends (at a given temperature) on water, fat and salt contents. To visualize the relationships among the individual sample variables, principle component analysis (PCA) was applied to the data given in Table 1 (water, fat and salt content) and the determined level of 3-MCPD. The relationships between the variables are given in Fig. 8. The right part of PC1 principally describes the variable ‘fat’, ‘salt’ and, to a smaller extent, ‘3-MCPD’. The upper part of PC2 describes the variable ‘water’ and to a smaller extent ‘3-MCPD’. It can be seen that the variables ‘fat’ and ‘salt’ positively correlate with 3-MCPD (in other words, the more fat or salt is in the sample the more 3-MCPD forms). Water does not seem to have much effect but the dependence of 3-MCPD on water is not simple as it reaches a maximum (Fig. 2).

The relationships between the individual samples are given in Fig. 9. The salami sample which has the highest content of 3-MCPD is situated along the positive part of PC1 as it also has the highest content of fat and salt. The sample of cheese is situated along the positive part of PC2 as it has high content of fat and salt and produces relatively high levels of 3-MCPD. Biscuits and crackers are clustered together as they have similar chemical composition. Their composition negatively correlates with water, fat, salt and 3-MCPD.

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